Figure 1

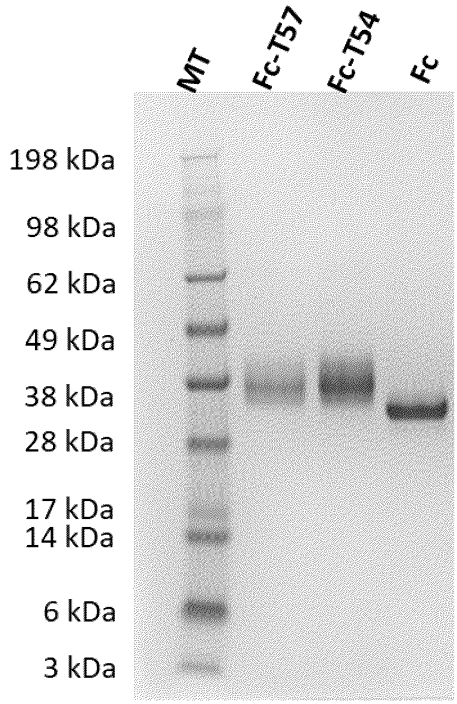


Figure 2

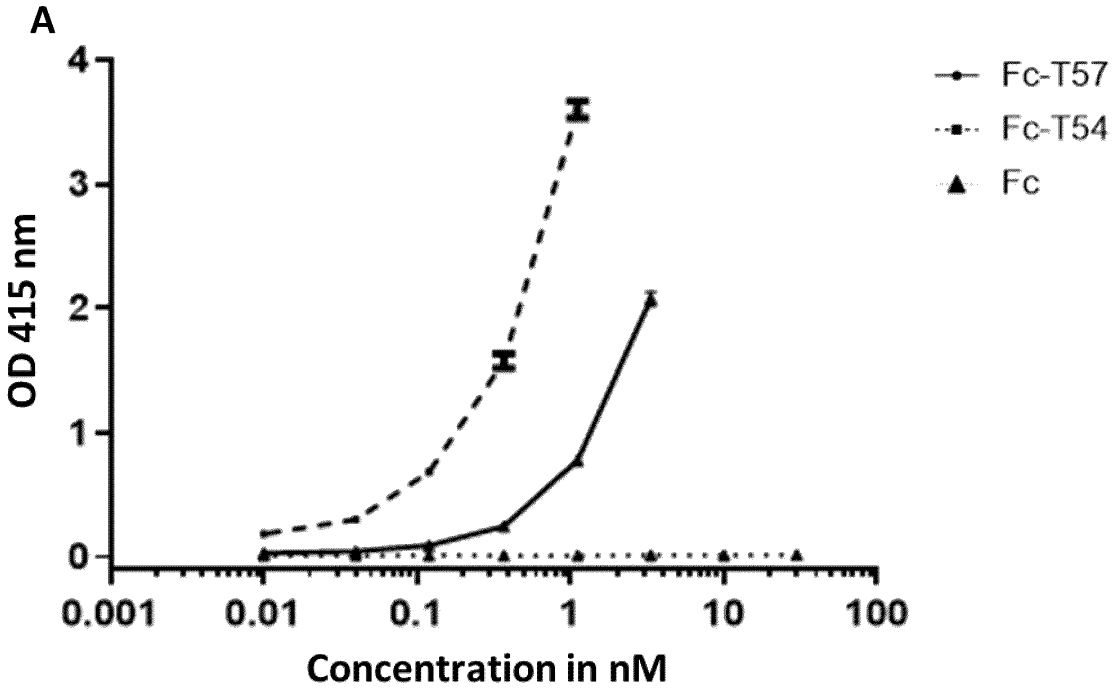
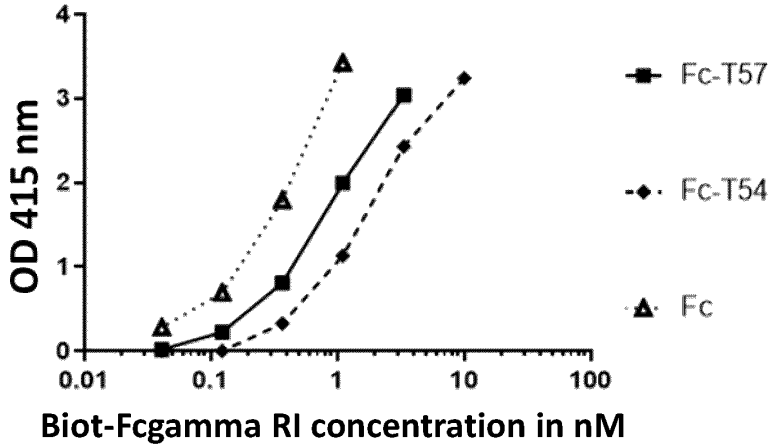


Figure 2 (continuation)

B



C

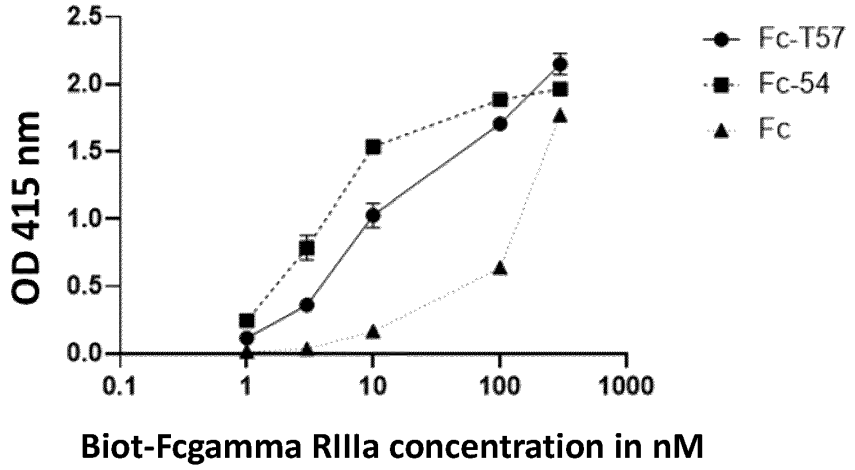
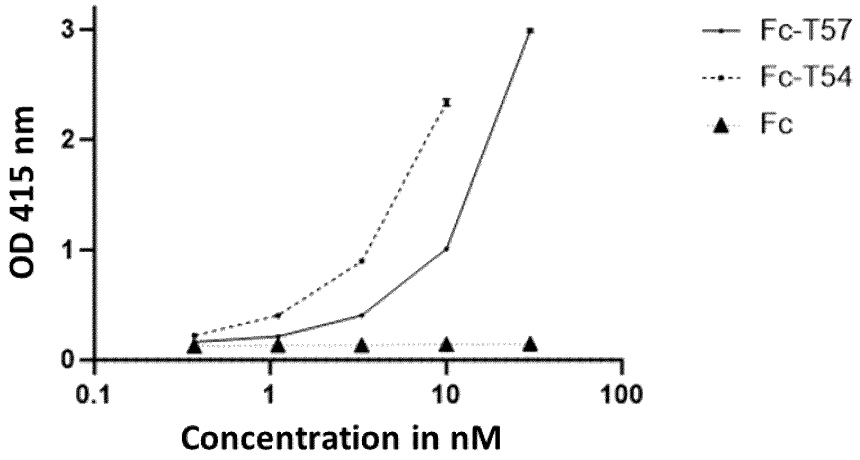


Figure 2 (continuation)

D



E

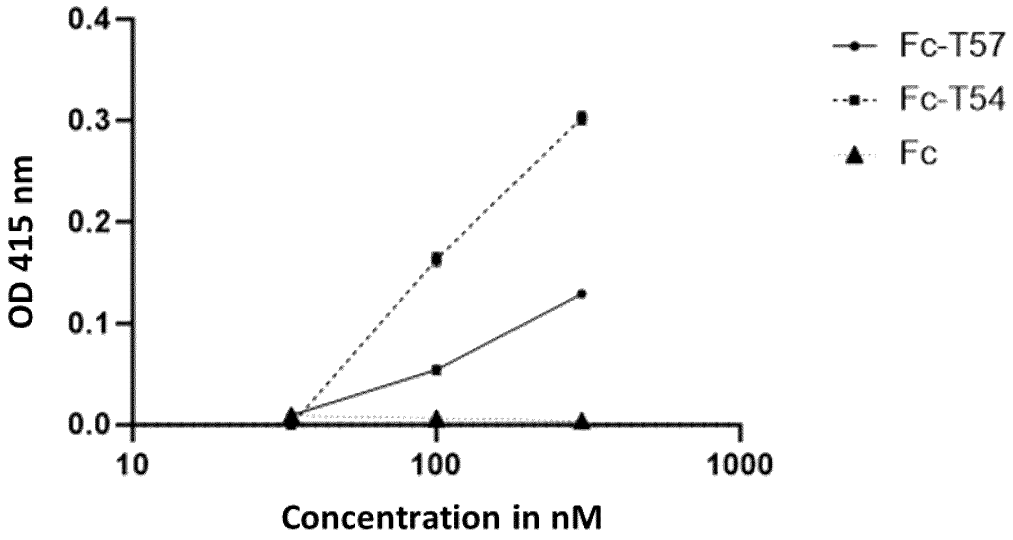


Figure 3

A

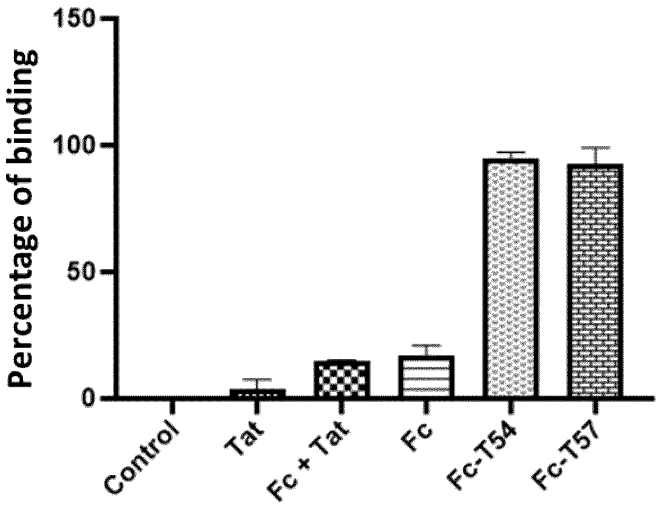
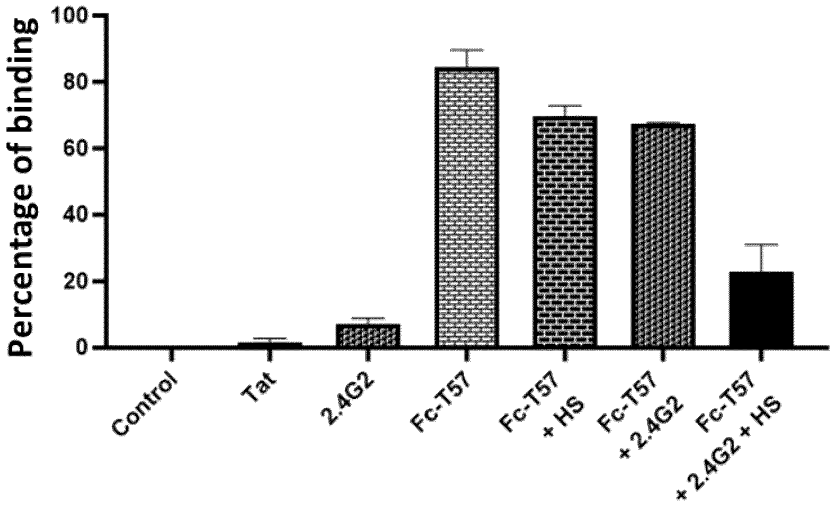

Percentage of binding
150
100
50
0
Control
Tat
Fc + Tat
Fc
Fc-T54
Fc-T57

Figure 3 (continuation)

B



C

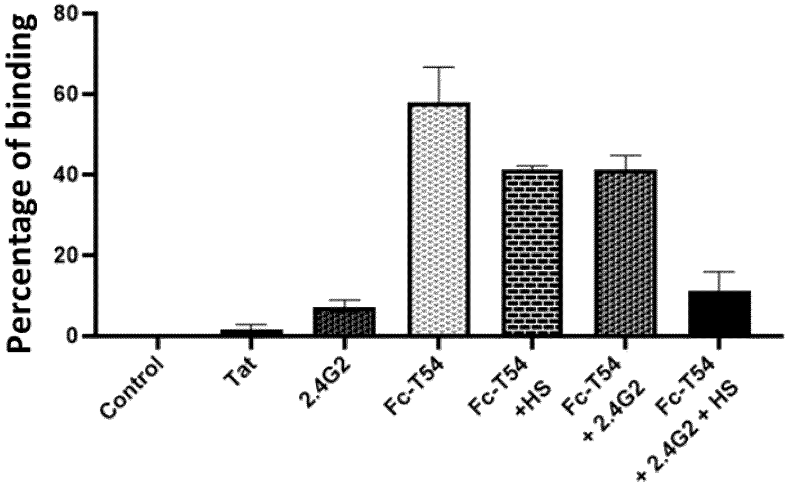


Figure 4

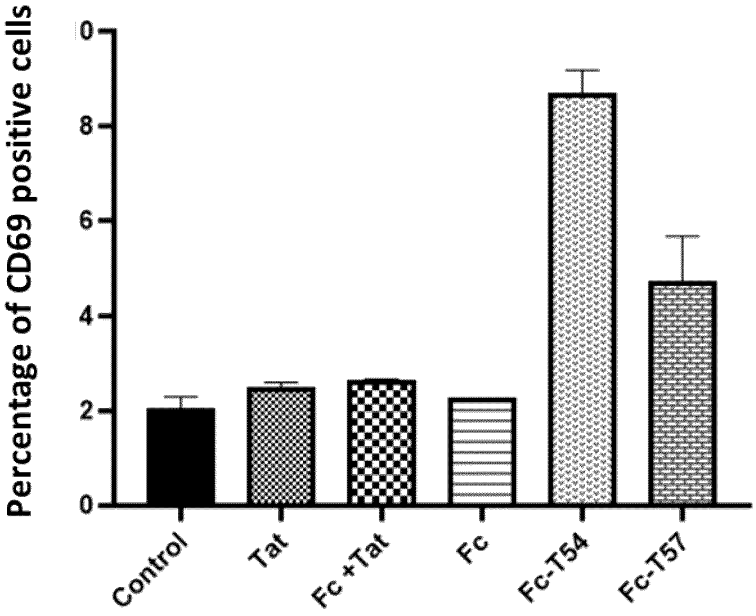
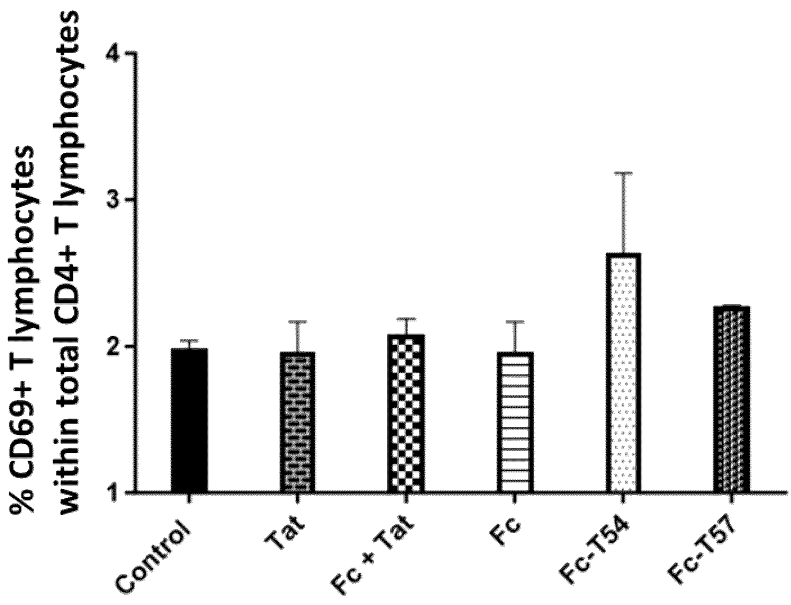

Percentage of CD69 positive cells
0
2
4
6
8
0
Control
Tat
Fc+Tat
Fc
Fc-T54
Fc-T57

Figure 5

A


% CD69+ T lymphocytes
within total CD4+ T lymphocytes
4
3
2
1
Control
Tat
Fc + Tat
Fc
Fc-T54
Fc-T57

B

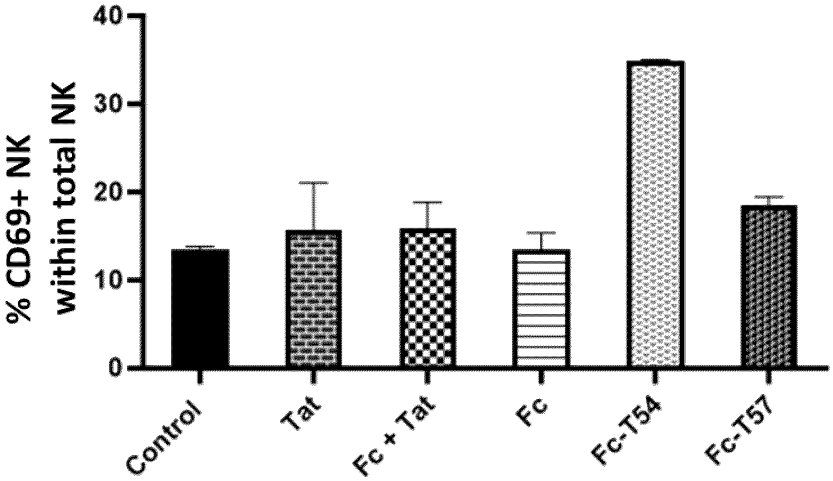

% CD69+ NK
within total NK
40
30
20
10
0
Control
Tat
Fc + Tat
Fc
Fc-T54
Fc-T57

Figure 6

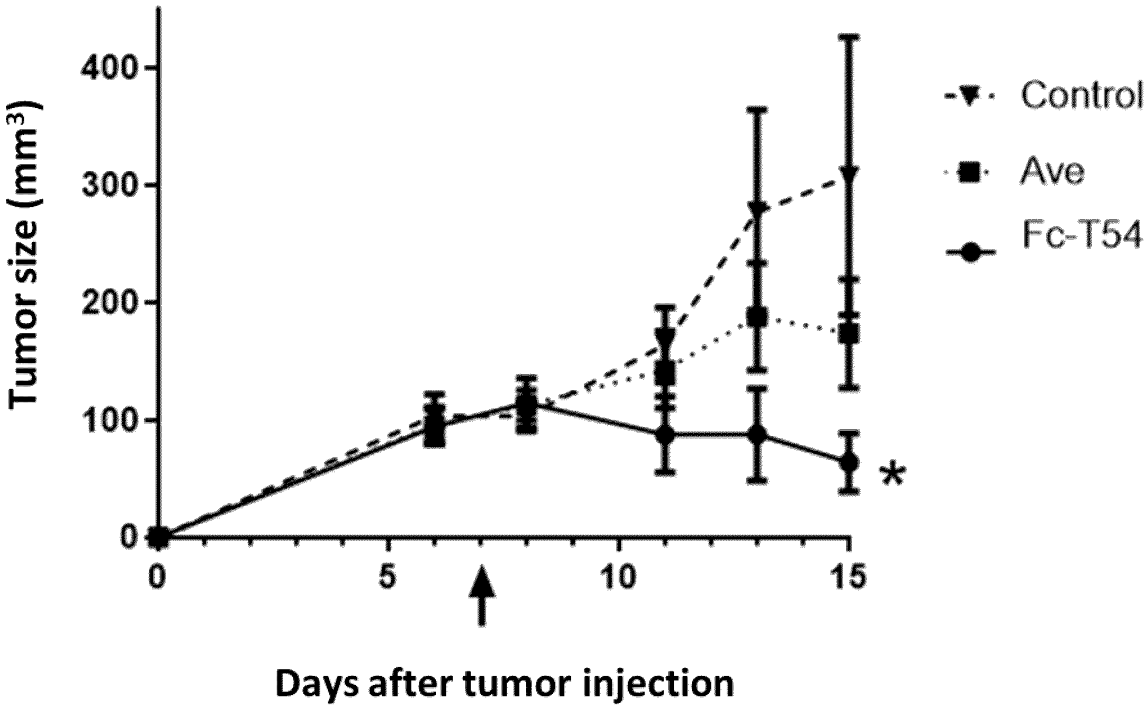

Tumor size (mm³)
400
300
200
100
0
0
5
10
15
*
Control
Ave
Fc-T54
Days after tumor injection

Figure 7

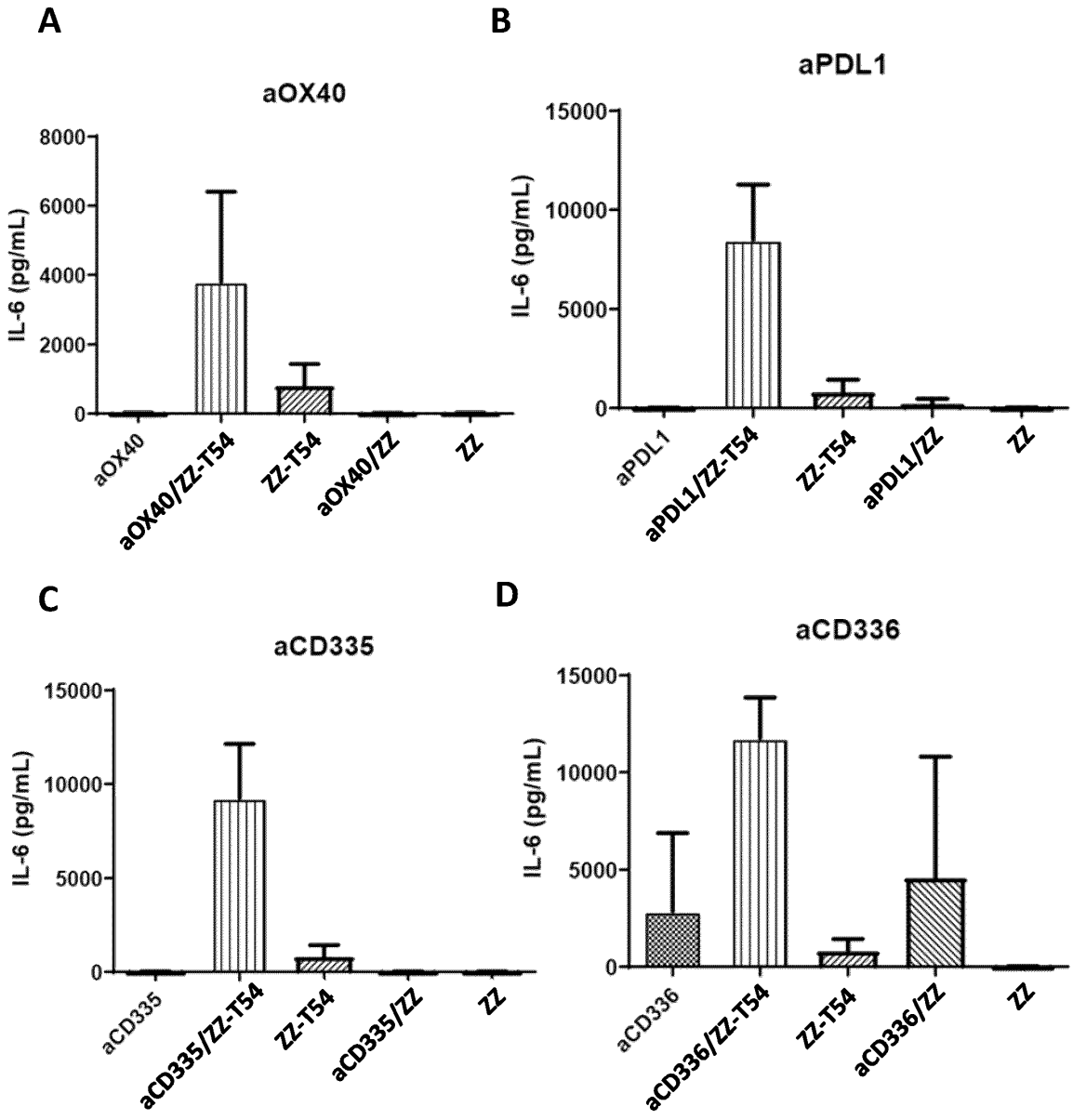
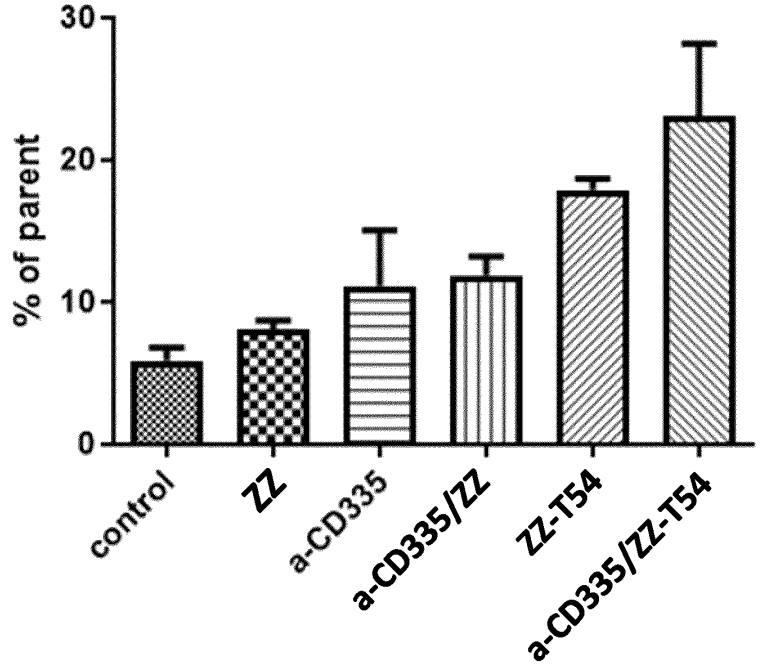

A
aOX40
IL-6 (pg/mL)
8000
6000
4000
2000
0
aOX40
aOX40/ZZ-T54
ZZ-T54
aOX40/ZZ
ZZ
B
aPDL1
IL-6 (pg/mL)
15000
10000
5000
0
aPDL1
aPDL1/ZZ-T54
ZZ-T54
aPDL1/ZZ
ZZ
C
aCD335
IL-6 (pg/mL)
15000
10000
5000
0
aCD335
aCD335/ZZ-T54
ZZ-T54
aCD335/ZZ
ZZ
D
aCD336
IL-6 (pg/mL)
15000
10000
5000
0
aCD336
aCD336/ZZ-T54
ZZ-T54
aCD336/ZZ
ZZ

Figure 8

A


30
20
10
0
% of parent
control
ZZ
a-CD335
a-CD335/ZZ
ZZ-T54
a-CD335/ZZ-T54

B

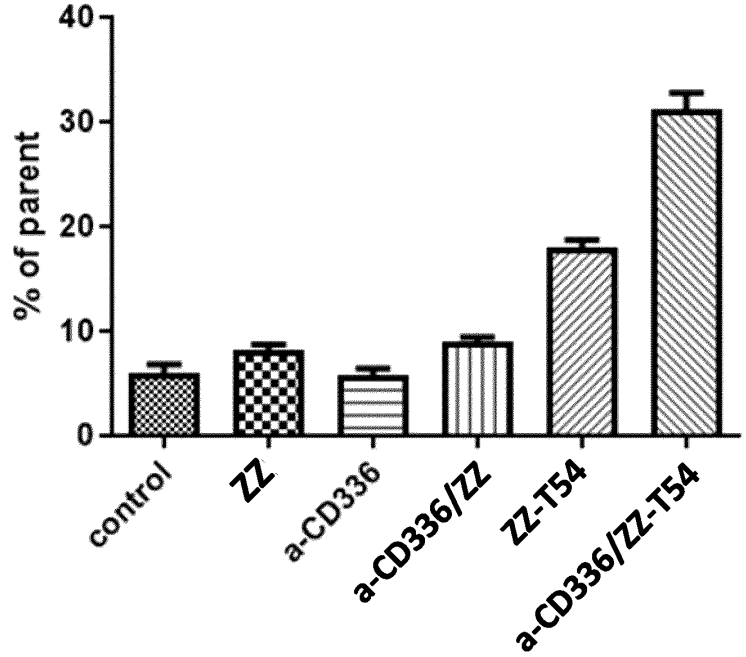

40
30
20
10
0
% of parent
control
ZZ
a-CD336
a-CD336/ZZ
ZZ-T54
a-CD336/ZZ-T54

Figure 9

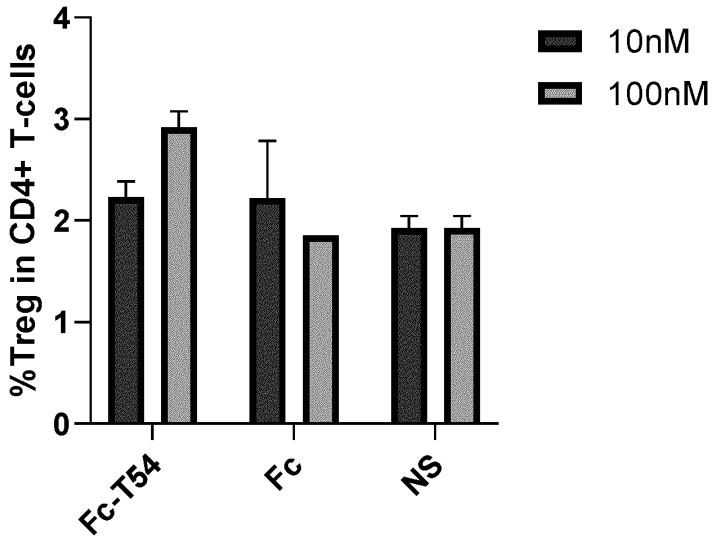


Figure 10

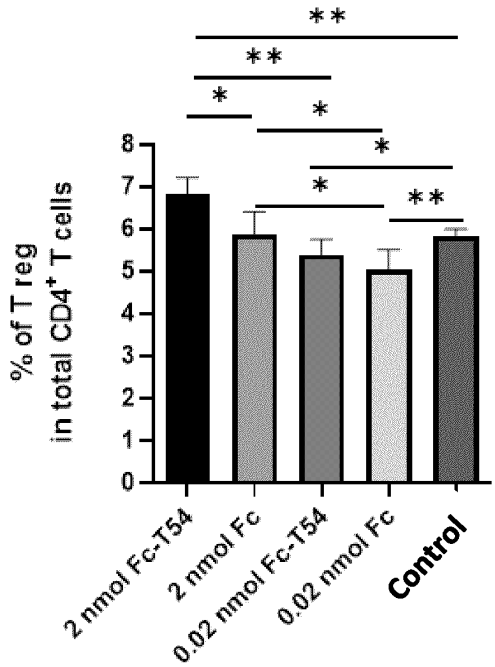


Figure 11

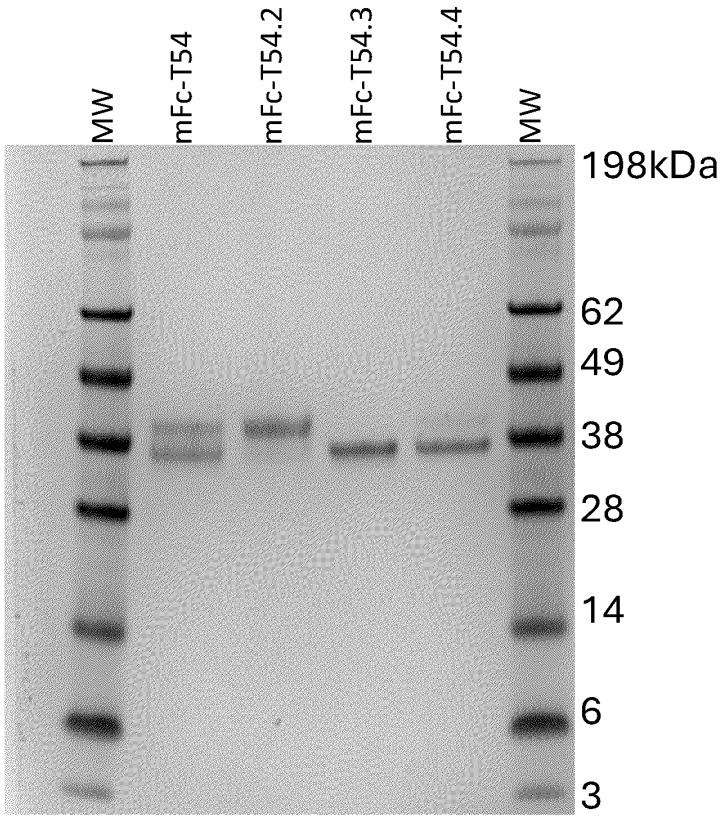


Figure 12

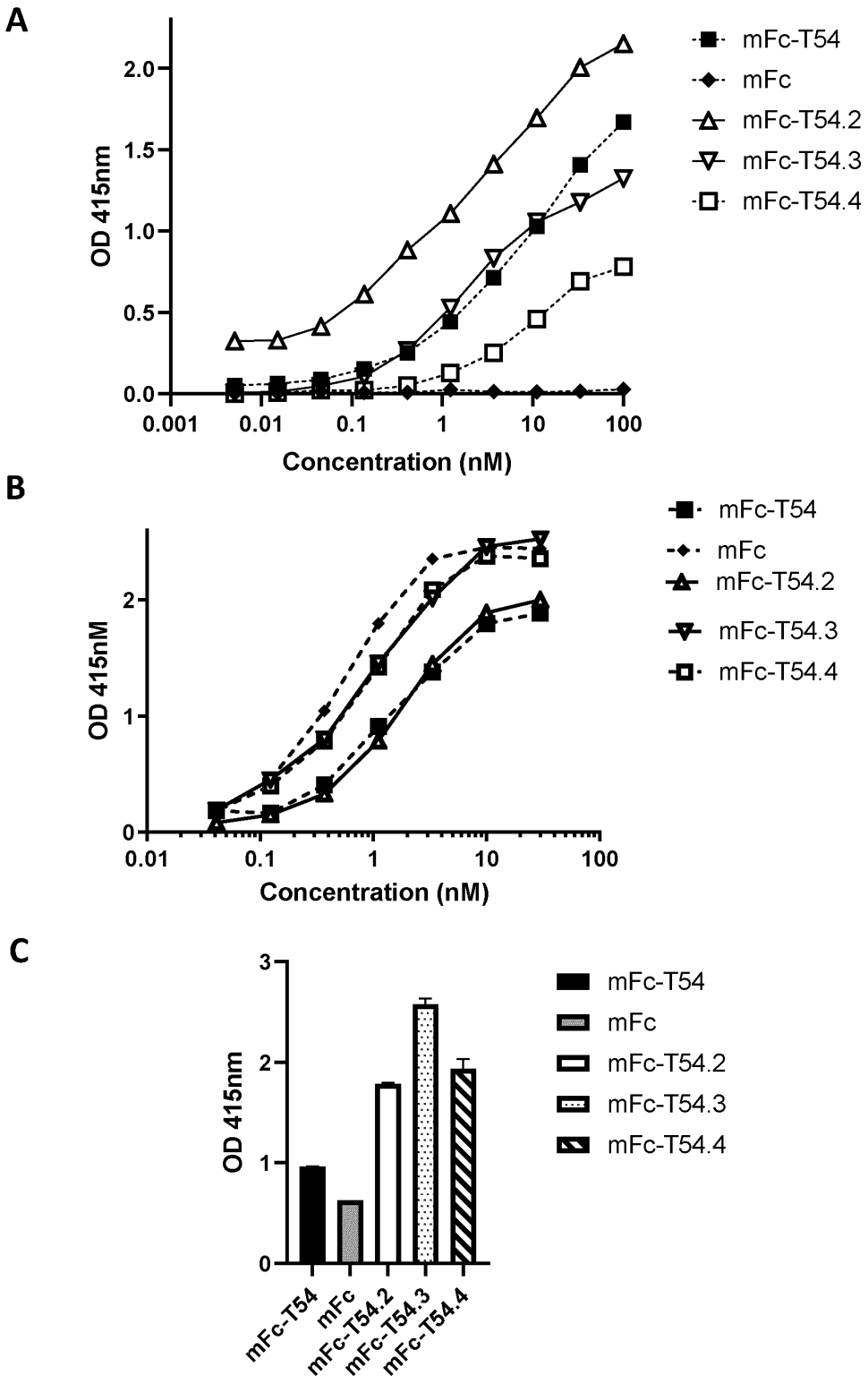
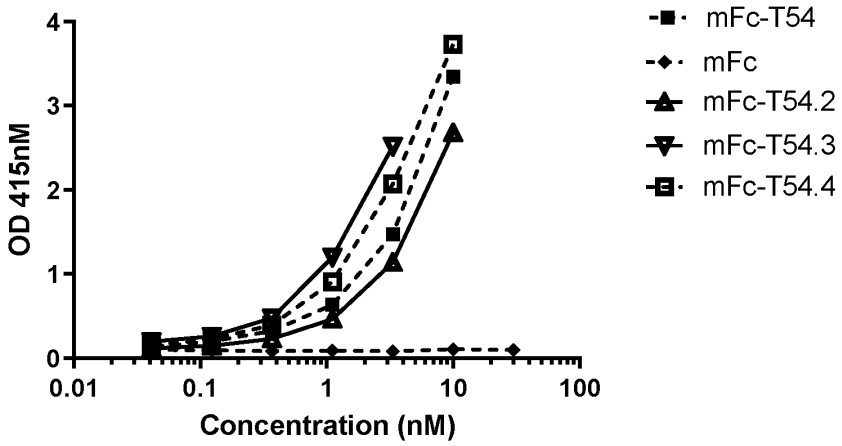

A
OD 415nm
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
100
Concentration (nM)
mFc-T54
mFc
mFc-T54.2
mFc-T54.3
mFc-T54.4
B
OD 415nM
2
1
0
0.01
0.1
1
10
100
Concentration (nM)
mFc-T54
mFc
mFc-T54.2
mFc-T54.3
mFc-T54.4
C
OD 415nm
3
2
1
0
mFc-T54
mFc
mFc-T54.2
mFc-T54.3
mFc-T54.4
mFc-T54
mFc
mFc-T54.2
mFc-T54.3
mFc-T54.4

Figure 12 (continuation)

D



E

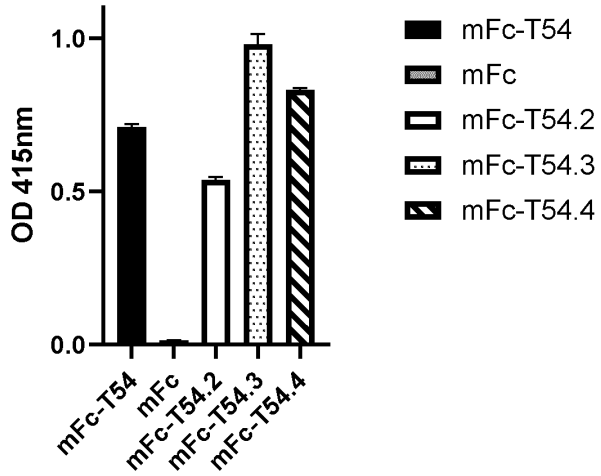
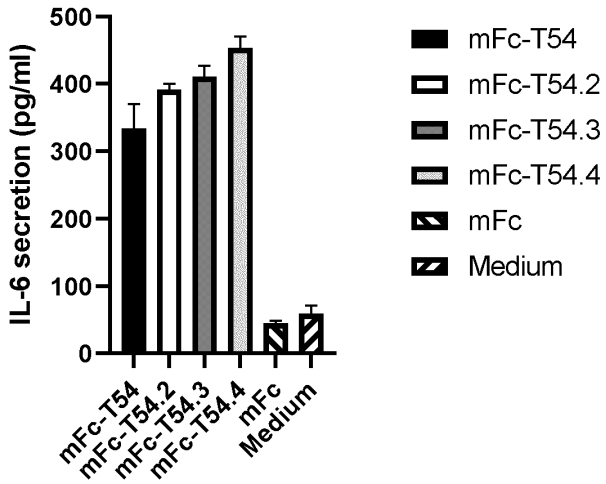


Figure 13

A


IL-6 secretion (pg/ml)
500
400
300
200
100
0
mFc-T54
mFc-T54.2
mFc-T54.3
mFc-T54.4
mFc
Medium
mFc-T54
mFc-T54.2
mFc-T54.3
mFc-T54.4
mFc
Medium

B

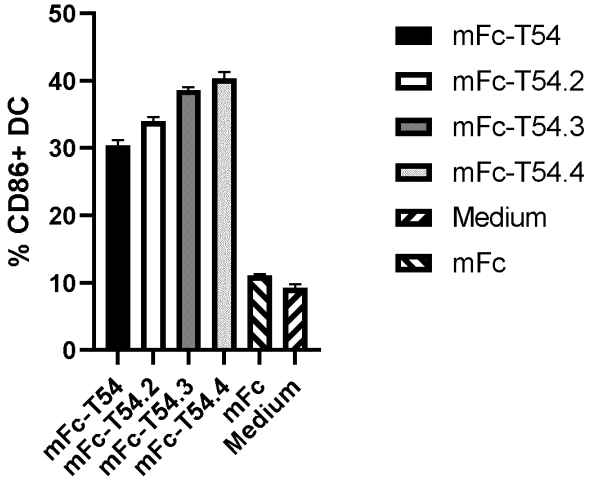

% CD86+ DC
50
40
30
20
10
0
mFc-T54
mFc-T54.2
mFc-T54.3
mFc-T54.4
mFc
Medium
mFc-T54
mFc-T54.2
mFc-T54.3
mFc-T54.4
Medium
mFc

Figure 14

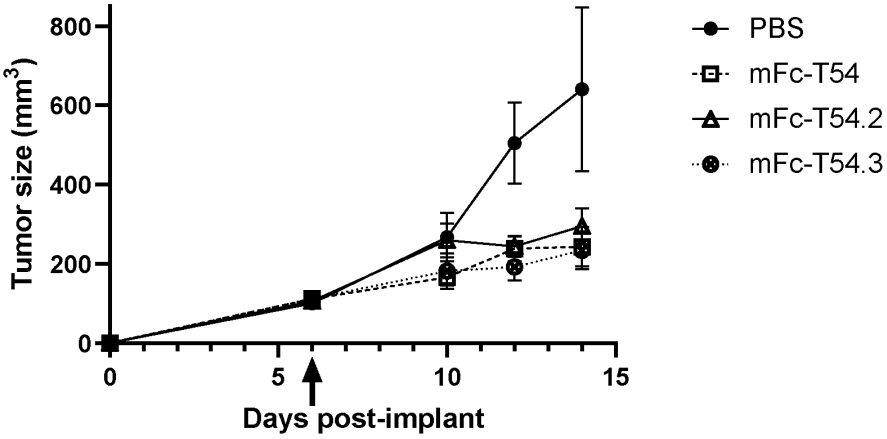
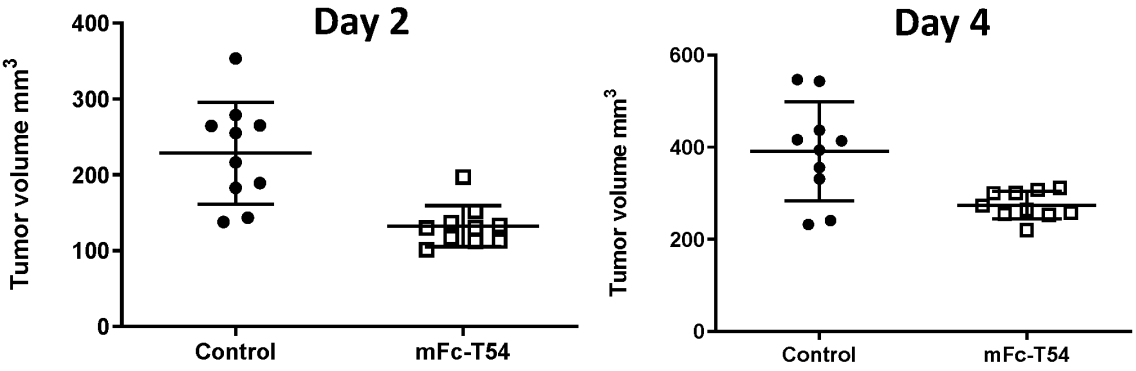


Figure 15

A



B

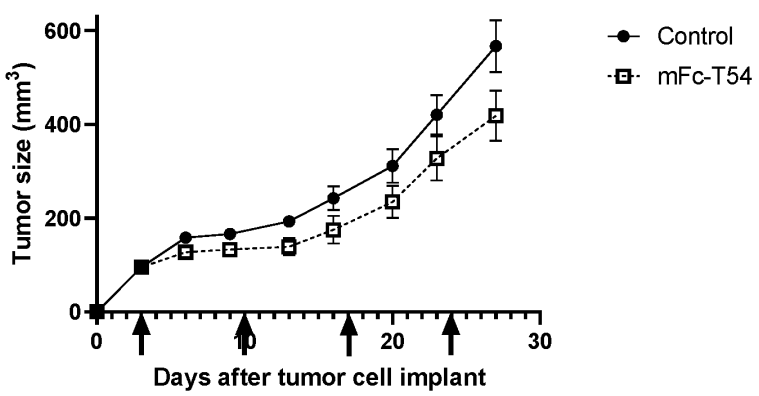
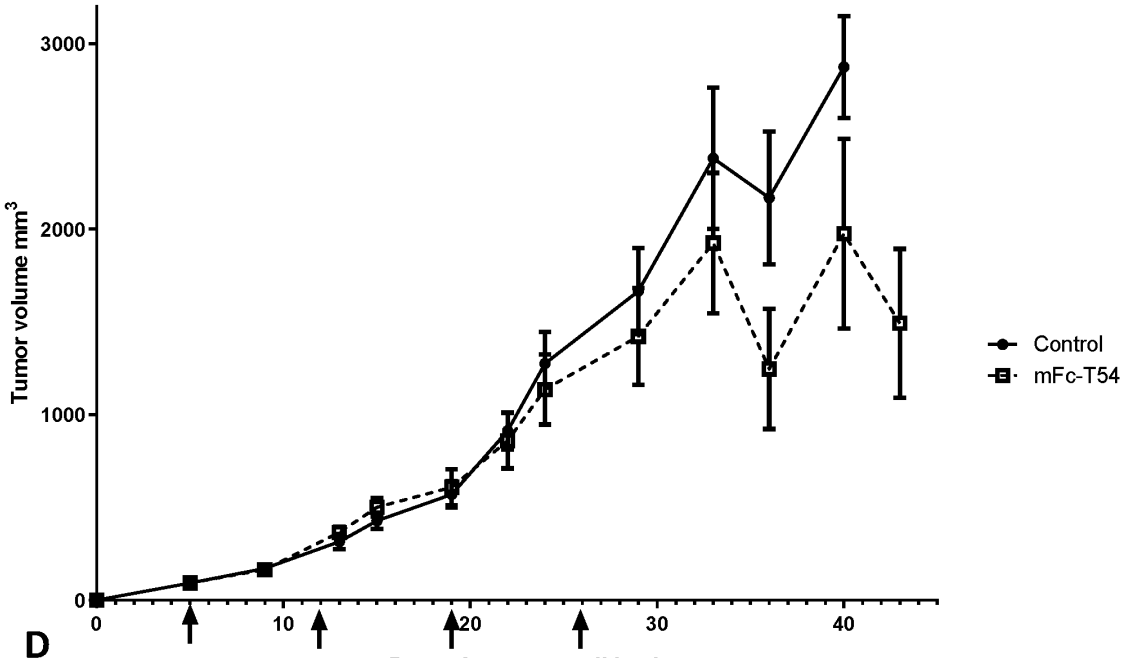


Figure 15 (continuation)

C



D

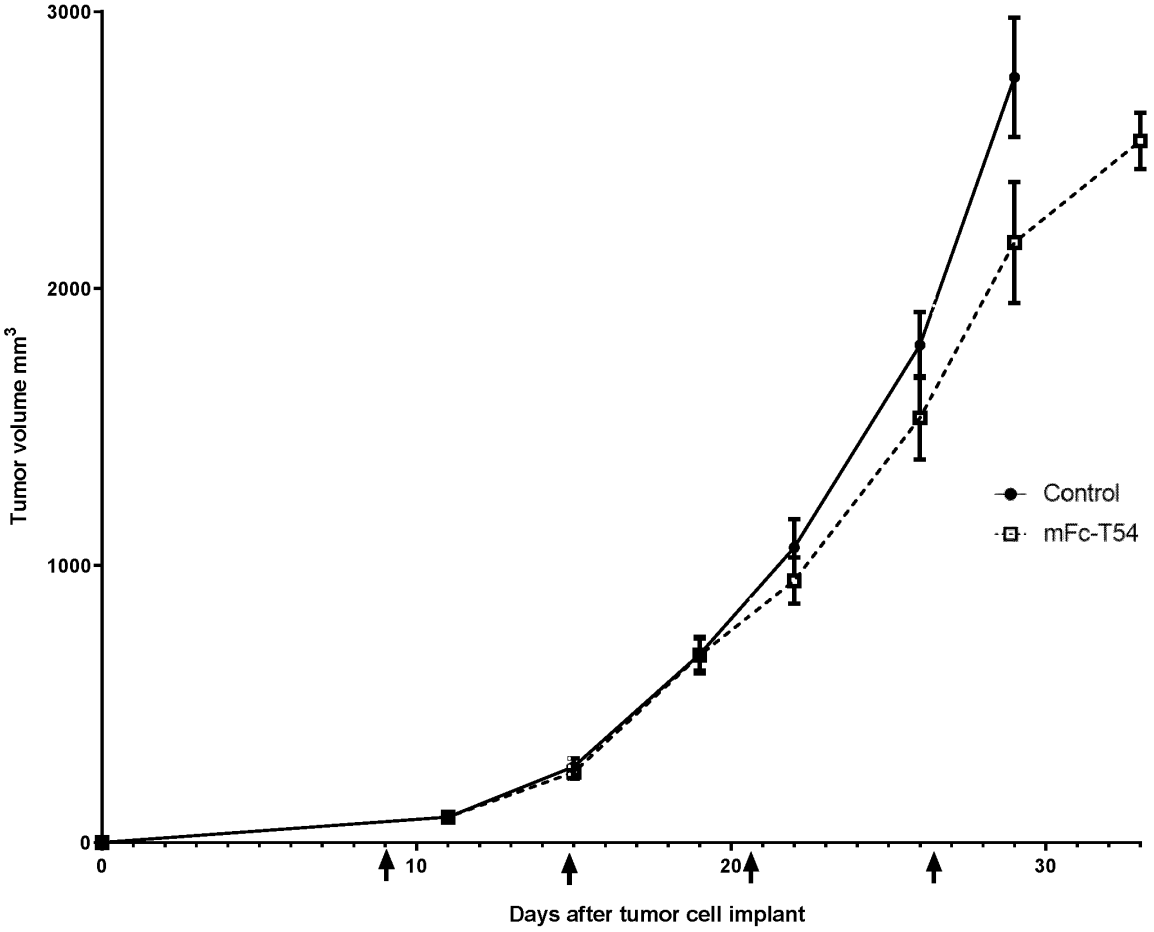


Figure 15 (continuation)

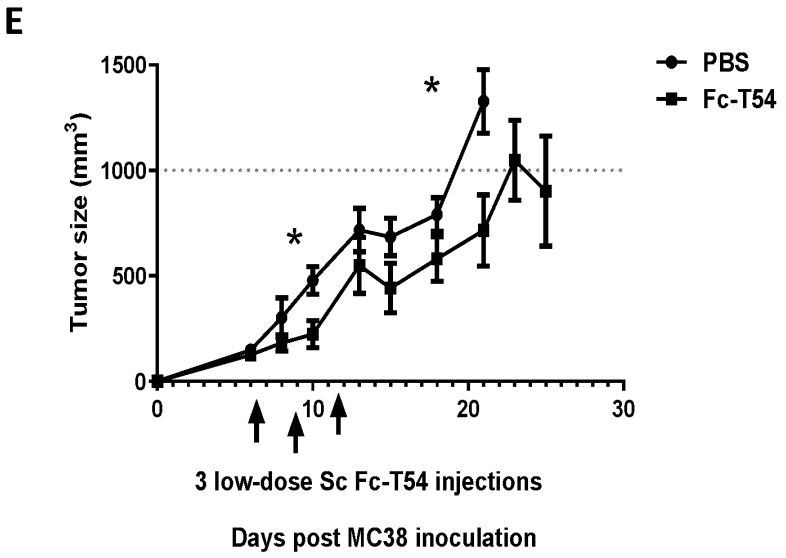


Figure 16

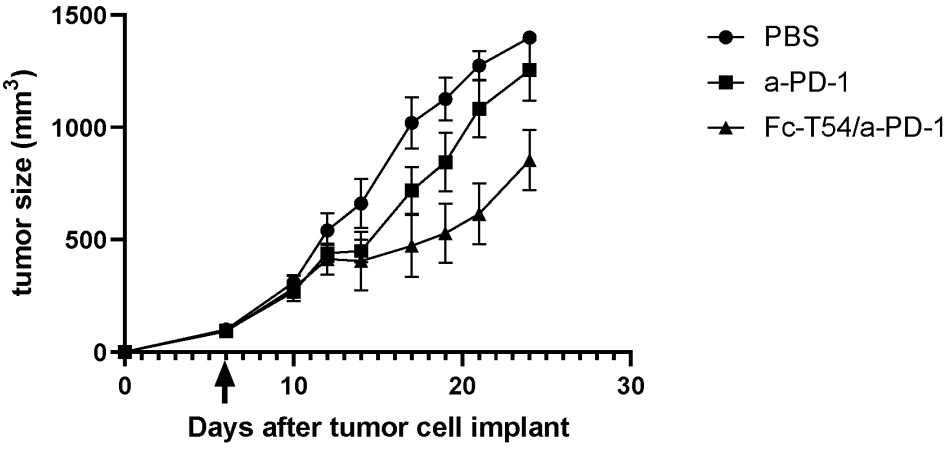
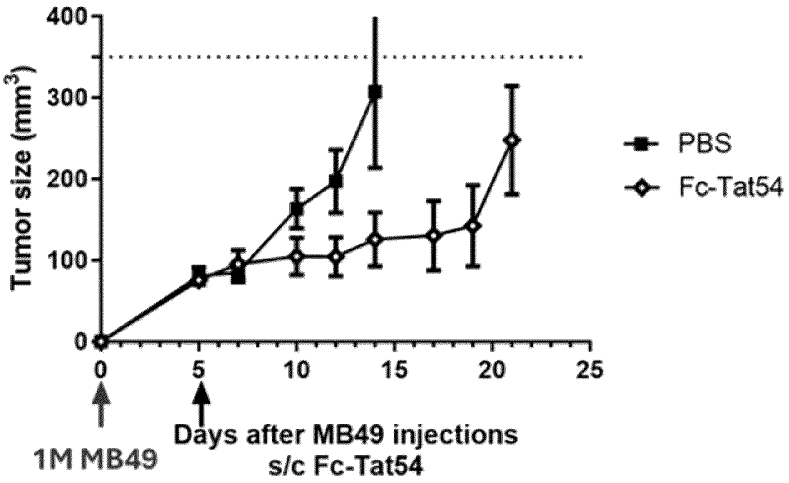


Figure 17

A


Tumor size (mm3)
400
300
200
100
0
0
5
10
15
20
25
1M MB49
Days after MB49 injections
s/c Fc-Tat54
PBS
Fc-Tat54

B

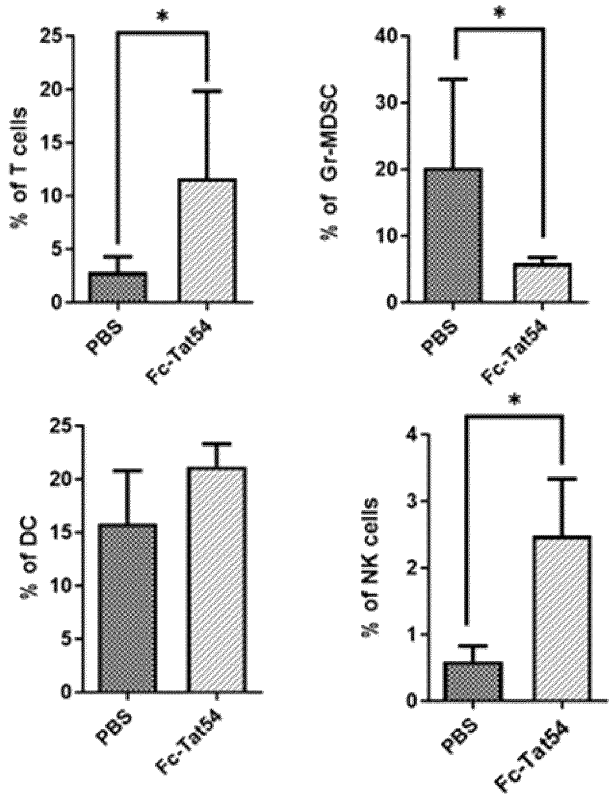
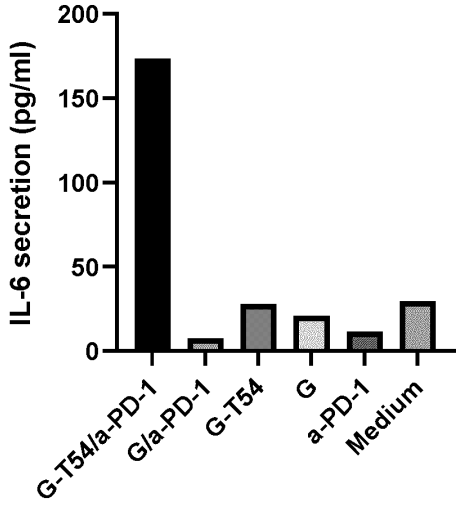

% of T cells
% of Gr-MDSC
% of DC
% of NK cells
PBS
Fc-Tat54
*

Figure 18


IL-6 secretion (pg/ml)
200
150
100
50
0
G-T54/a-PD-1
G/a-PD-1
G-T54
G
a-PD-1
Medium